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H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in *Arabidopsis*

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SUMMARY

Plants are highly sensitive to temperature and can perceive a difference of as little as 1°C. How temperature is sensed and integrated in development is unknown. In a forward genetic screen in *Arabidopsis*, we have found that nucleosomes containing the alternative histone H2A.Z are essential to perceiving ambient temperature correctly. Genotypes deficient in incorporating H2A.Z into nucleosomes phenocopy warm grown plants, and show a striking constitutive warm temperature transcriptome. We show that nucleosomes containing H2A.Z display distinct responses to temperature in vivo, independently of transcription. Using purified nucleosomes, we are able to show that H2A.Z confers distinct DNA-unwrapping properties on nucleosomes, indicating a direct mechanism for the perception of temperature through DNA-nucleosome fluctuations. Our results show that H2A.Z-containing nucleosomes provide thermosensory information that is used to coordinate the ambient temperature transcriptome. We observe the same effect in budding yeast, indicating that this is an evolutionarily conserved mechanism.

INTRODUCTION

Sessile organisms such as plants continually sense environmental conditions to adapt their growth and development. Temperature varies both diurnally, which is important for entraining the clock (Michael et al., 2008; Salome and McClung, 2005), as well as seasonally, providing information for the timing of reproduction (Heggie and Halliday, 2005; Samach and Wigge, 2005; Sung and Amasino, 2005). Extremes of temperature represent a significant stress for plants and are a major factor limiting global plant distribution (Mittler, 2006). The sensitivity of plants to small changes in temperature is highlighted by significant changes in flowering time (Fitter and Fitter, 2002) and distributions of wild plants that have occurred in the last 100 years due to climate change (Kelly and Goulden, 2008; Lenoir et al., 2008; Willis et al., 2008). Projected increases in mean global temperature as well as extremes of temperature in the next 100 years are significantly larger than what has occurred so far, suggesting significant future disruption to wild plants and crops.

Arabidopsis thaliana has a highly plastic life history, and a genetically informed photothermal model is able to explain most of the variation in flowering time for plants grown at different seasons (Wilczek et al., 2009). Acceleration of flowering in response to higher temperature requires genes in the autonomous pathway (Blazquez et al., 2003) and is dependent on increasing expression of *FLOWERING LOCUS T (FT)* (Balasubramanian et al., 2006; Cerdan and Chory, 2003; Halliday et al., 2003; Lee et al., 2007). We performed a forward screen to identify genes required for the control of transcription in response to ambient temperature.

We find that H2A.Z-containing nucleosomes represent the major node of regulation of the temperature transcriptome in plants. H2A.Z nucleosomes wrap DNA more tightly, which influences the ability of RNA polymerase (Pol) II to transcribe genes in response to temperature, suggesting a mechanism by which the transcriptome can be thermally regulated.

RESULTS

HSP70 Is an Output of the Ambient Temperature-Sensing Pathway

To identify the major ambient temperature responses in seedlings, we analyzed the transcriptomes of 12-day-old plants grown at 12°C and shifted to 27°C. We found that 2454 genes are upregulated at least 2-fold under these conditions and 2880 genes are 2-fold downregulated (the ambient temperature transcriptome; Figure 1A). As in similar studies, there was not a significant induction of stress markers, suggesting that this temperature range is not causing heat stress (Balasubramanian et al., 2006). We found that *HSP70* (At3g12580) is strongly upregulated at higher temperature (Figure 1B). Although this transcriptome is characterized by a response to ambient temperature change, we wanted to determine if any of these genes were also responsive to differences in constant growth temperature since the transcriptional output of a thermosensory pathway should be different at various constant temperatures.

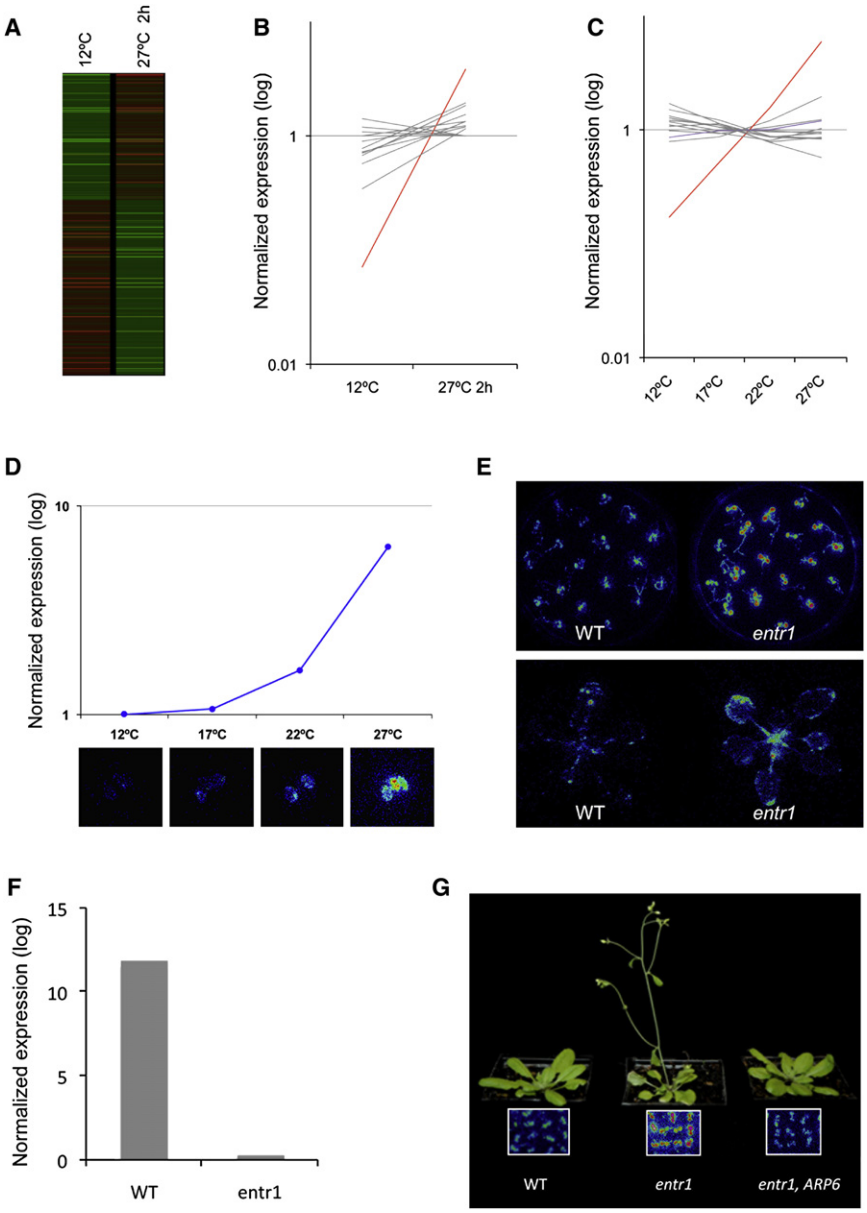

Figure 1. *HSP70* Expression Is an Output of the Ambient Temperature-Sensing Pathway

(A) Transcript profiling experiments show a robust transcriptional response to changes in ambient temperature.

(B) *HSP70* (red) is induced strongly with increasing ambient temperature. In contrast, the other members of the *HSP70* family genes (gray) require a heat stress to be upregulated.

(C) *HSP70* (red) has a uniform linear expression pattern at various constant growth temperatures, in contrast to the rest of the *HSP70* family (gray). *HSP70* is therefore an excellent output of the thermosensory pathway over a wide temperature range.

(D) *HSP70::LUC* in *Arabidopsis* mimics the endogenous *HSP70* expression pattern in response to temperature. *LUC* transcript analysis and live luciferase imaging of plants shifted to 17°C, 22°C, and 27°C for 2 hr from 12°C show a temperature-dependent LUC expression and luciferase activity. *LUC* transcript levels were normalized to *UBQ10*.

(E) Identification of *entr1* through a forward genetic screen as a mutant with enhanced temperature response at the seedling stage (upper panel); this phenotype is present throughout the life cycle. Lower panel shows the *HSP70::LUC* expression in adult wild-type and *entr1* plants.

(F) Transcript profiling analysis showing *ARP6* gene expression in wild-type (WT) and *entr1*. *ARP6* transcripts were absent in *entr1*. The bar represents normalized expression levels from the microarray experiment.

(G) Complementation of *entr1*: a binary construct containing the *ARP6* genomic fragment including the native promoter and coding regions (P_{ARP6}::*ARP6*) completely rescued the *HSP70*::*LUC* as well as the developmental phenotypes of *entr1*.

We therefore analyzed the behavior of the induced ambient temperature transcriptome genes in publicly available data for plants grown at 12°C, 17°C, 22°C, and 27°C (NASCARRAYS-147; Gould et al., 2006). We found that *HSP70* is expressed at a level proportionate to the ambient temperature within this temperature range (Figure 1C). Although these experiments were performed on seedlings, *HSP70* also shows these expression dynamics in the adult plant (Balasubramanian et al., 2006). We therefore used a fusion of the *HSP70* promoter to Luciferase *(HSP70::LUC)* to monitor the activity of the endogenous gene nondestructively. *HSP70::LUC* recapitulates the expression of *HSP70* (Figure 1D), providing us with a dynamic and sensitive assay for temperature perception status in planta, independent of high temperature stress (Larkindale and Vierling, 2008; Sung et al., 2001).

ARP6 Is in the Ambient Temperature-Sensing Pathway that Controls Flowering

To identify genes required to control the ambient temperature transcriptome, we screened 2600 individual M2 families of fast neutron irradiated *HSP70::LUC* seedlings. Two mutations, *entr1* and *entr2 (enhanced temperature response1 and 2)*, displayed a constitutively higher *LUC* expression (Figure 1E). Genetic analysis using a complementation cross revealed that these mutations are allelic. Transcript-based cloning revealed that the *ARP6* transcript is absent in both *entr1* and *entr2* (Figure 1F). Transformation of *entr1* with a genomic fragment of *ARP6* is able to rescue both the *HSP70::LUC* expression level as well as the altered development of *entr1* (Figure 1G), confirming that it is a new *arp6* allele. We will refer to *entr1* and *entr2* as *arp6-10* and *arp6-11*, respectively. *Arabidopsis* mutants in *ARP6* have been identified in flowering time screens (Choi et al., 2005; Deal et al., 2005; Martin-Trillo et al., 2006). To determine if *ARP6* acts in the ambient temperature pathway for flowering, we analyzed flowering in *arp6-10*. Consistent with earlier reports, *arp6-10*

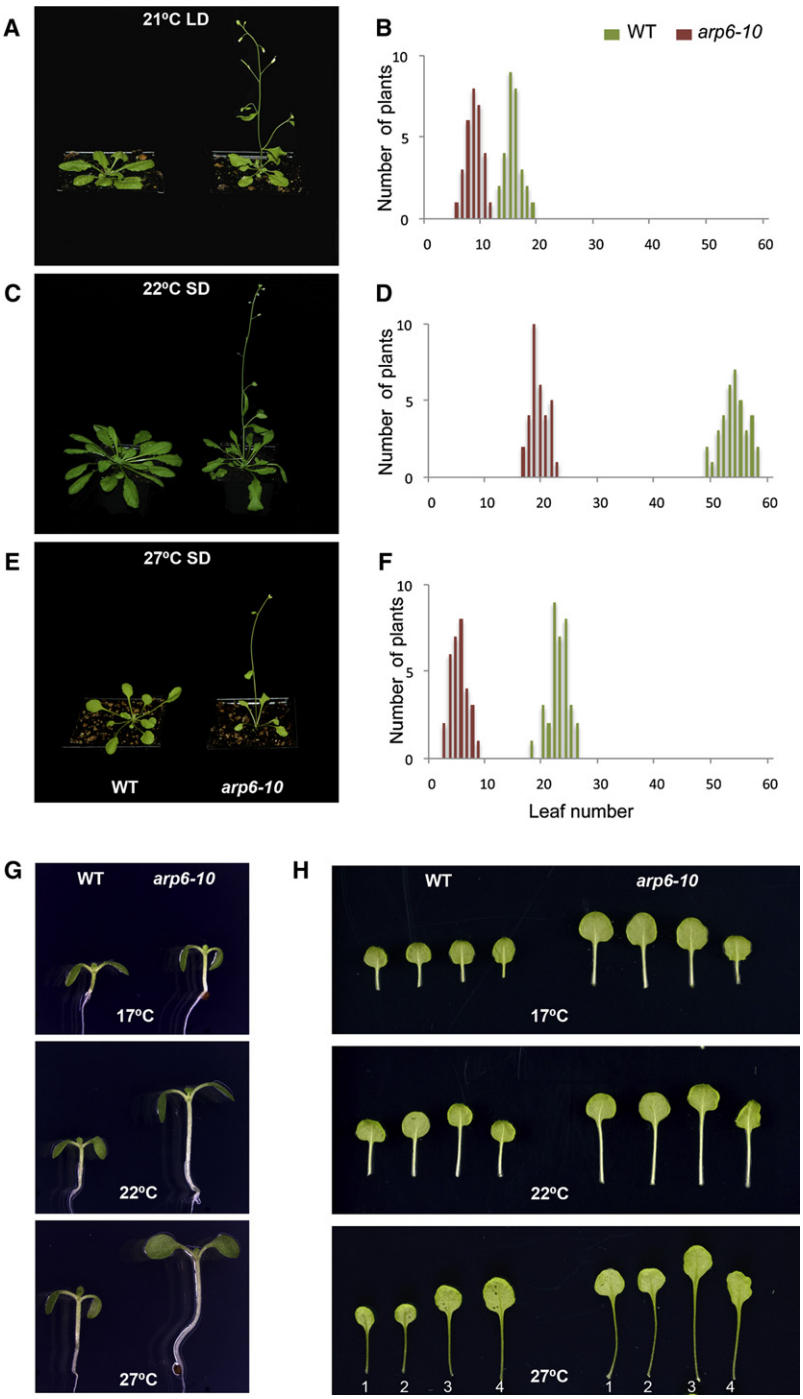

Figure 2. *arp6-10* Displays Developmental and Architectural Phenotypes of Warm Grown Plants

(A–D) *arp6-10* flowers significantly earlier than wild-type under long-day (A and B) and short-day conditions (C and D) at 22°C.

When grown in short days at 27°C (E and F), *arp6-10* shows a very strong thermal induction of flowering. In comparison, wild-type flowering time is similar to *arp6-10* at 22°C. (x axis shows the number of rosette leaves at the time of flowering, y axis shows the corresponding number of plants.)

(G and H) In addition to the flowering time phenotypes, *arp6-10* displays all the architectural responses of wild-type plants grown at high temperature. *arp6-10* seedlings at 17°C show hypocotyl (G) and petiole elongation (H) that is equivalent to the wild-type phenotype at higher temperatures. (Petiole elongation was analyzed on the first four true leaves of 20-day-old wild-type and *arp6-10* seedlings.)

See also Figure S1.

flowers earlier at 21°C in long and 22°C in short days (Figures 2A, 2B, 2C, and 2D). We then studied the response of *arp6-10* to 27°C in short days because the thermal induction of flowering is most pronounced in short days (Balasubramanian et al., 2006). Remarkably, *arp6-10* flowers with about five leaves in short days at 27°C (Figures 2E and 2F). *ARP6* therefore acts in the ambient temperature pathway that controls flowering.

ARP6 Controls Developmental Responses to Ambient Temperature Globally

Specific adaptive changes to plant architecture, including increases in hypocotyl growth and petiole elongation, occur in response to higher ambient temperature (Gray et al., 1998; Koini et al., 2009). We analyzed these traits to see if *arp6-10* exhibits a global high-temperature response in its architecture as well as *HSP70* expression and flowering time. We find that architecture responses are strongly enhanced in the *arp6* background (Figures 2G and 2H and Figure S1 available online), such that *arp6* plants grown at 17°C exhibit greater hypocotyl elongation and petiole growth than wild-type plants grown at 22°C, with an equivalent difference between 22°C and 27°C. These phenotypes have been shown to be dependent on the PIF4 transcription factor (Koini et al., 2009), so it is not surprising that we still observe a temperature-induced difference, even in the *arp6-10* mutant. A functional *ARP6* is required, however, for controlling the correct level of expression of these phenotypes. A longstanding observation in plant biology is that thermal time is a key measure for the rate of transition through the developmental phases, and that phase transition is accelerated by higher ambient temperature (Poethig, 2003). In addition to the flowering time acceleration in *arp6-10*, we observe a more rapid juvenile-to-adult transition (data not shown), consistent with earlier studies (Martin-Trillo et al., 2006). Thus *arp6-10* causes a specific upregulation of all the developmental decisions known to be regulated by temperature that we have examined. Plants deficient in *ARP6* therefore display a constitutive warm temperature developmental program.

The Ambient Temperature Transcriptome Is Globally Misregulated in *arp6*

To determine if the global misregulation of developmental responses in the absence of *ARP6* occurs at the transcriptional level, we compared the ambient temperature transcriptomes of *arp6-10* and wild-type. While 2454 genes are upregulated 2-fold or more on shifting from 12°C to 27°C in wild-type (Figure 3A, gray bars), we observe that this ambient temperature transcriptome is consistently expressed in *arp6-10*, even at 12°C (Figure 3A, blue bars). Interestingly, this effect reflects more than a constitutive upregulation of gene expression per se given that the 2880 genes that are downregulated at least 2-fold on increasing temperature in wild-type (Figure 3B, gray bars) are also constitutively repressed in *arp6-10*. Thus, transcriptionally, the *arp6-10* mutant at 12°C resembles a plant at a higher temperature. In a reciprocal analysis, transcripts that are 2-fold more highly expressed at 12°C in *arp6-10* compared to wild-type (Figure 3C, blue bars) are strongly induced by higher ambient temperature in wild-type, whereas those genes that are repressed in *arp6-10* compared to wild-type at 12°C (Figure 3D, blue bars), are transcriptionally repressed by higher temperature in wild-type (Figure 3D, gray bars). These results show that in the absence of *ARP6,* the ambient temperature-regulated genes are constitutively misexpressed. To determine what proportion of the entire ambient temperature transcriptome is *ARP6* dependent, we compared the change of gene expression in response to higher ambient temperature in wild-type with the change in expression resulting from *arp6-10* at constant temperature. Remarkably, there is a strong correlation for the entire transcriptome: a functional *ARP6* allele accounts for almost half of the ambient temperature transcript responses ($R^2 = 0.466$, $p < 0.001$). *ARP6* is therefore necessary for coordinating the transcriptome in response to ambient temperature.

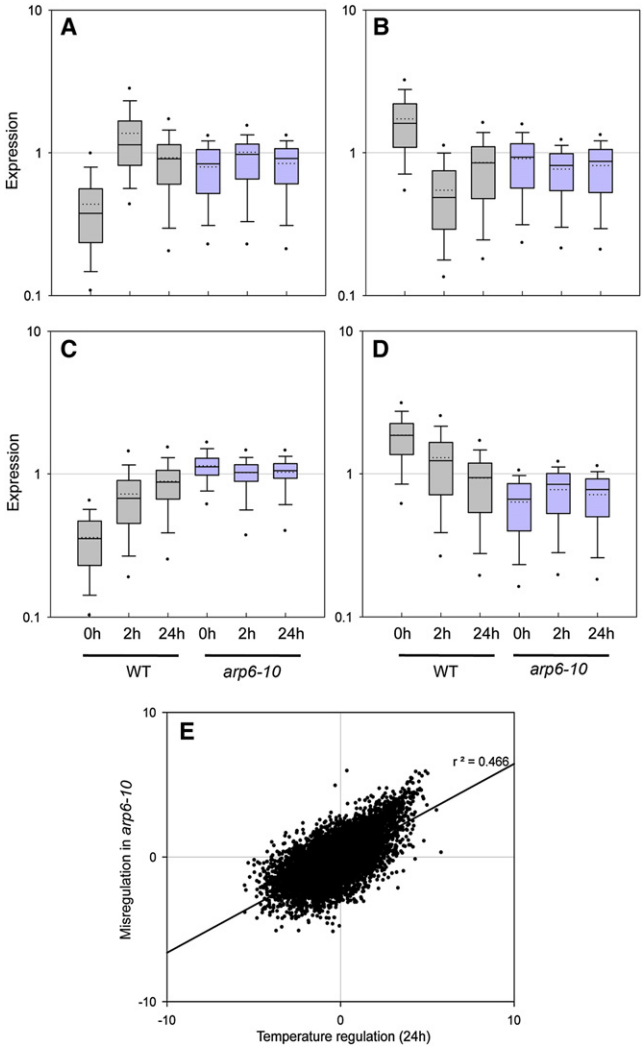

Figure 3. The Temperature Transcriptome Is Globally Misregulated in *arp6-10*

Genes that are 2-fold up- (A) or downregulated (B) in wild-type seedling within 2 hr of shifting to 27°C are constitutively repressed or activated in the *arp6-10* background (blue bars). Similarly genes that are 2 fold up- (C) or downregulated (D) in *arp6-10* compared to the wild-type at 12°C (0 hr) were down- or upregulated in the wild-type upon shift to 27°C. Median and mean values are represented, respectively, by the solid and dotted horizontal lines. Whiskers represent lowest and highest nonoutlier values. Outlier values are represented by 5th and 95th percentiles (dots).

(E) Comparison of *arp6-10*-induced genome-wide transcriptional changes with responses to increasing temperature from 12°C to 27°C in wild-type. Whole-genome scatter plot analysis of expression log ratios. x axis: comparison of wild-type after 24 hr at 27°C with seedlings kept at 12°C. y axis: comparison of *arp6-10* grown at 12°C with wild-type at 12°C. The *arp6-10* mutation accounts for nearly half ($R^2 = 0.466$) of the changes in the transcriptome in response to an increase in ambient temperature.

H2A.Z Occupancy Changes with Temperature

ARP6 encodes a subunit of the SWR1 complex that is conserved among eukaryotes and is necessary for inserting the alternative histone H2A.Z into nucleosomes in place of H2A (Deal et al., 2007; Kobor et al., 2004; Krogan et al., 2003; Mizuguchi et al., 2004). There are several H2A.Z family members in *Arabidopsis*, and we find that the *hta9 hta11* double mutant phenocopies *arp6-10*, showing the early flowering and architectural responses as well as increased *HSP70* expression. This indicates that the temperature responses of *arp6-10* result from a failure to incorporate H2A.Z-containing nucleosomes into the genome. Global analyses in yeast, *Drosophila*, *C. elegans*, *Arabidopsis*, and mammalian cells have revealed that H2A.Z-containing nucleosomes are especially enriched at the +1 position near the transcriptional start site (Creyghton et al., 2008; Mavrich et al., 2008; Raisner et al., 2005; Whittle et al., 2008; Zhang et al., 2005; Zilberman et al., 2008). H2A.Z has been implicated in maintaining promoters of quiescent genes in a poised state ready for appropriate transcription (Li et al., 2005). A possible explanation for the central role of *ARP6* in regulation of the temperature transcriptome is that H2A.Z-containing nucleosomes confer a temperature dependence on transcription. To test if H2A.Z dynamics change in response to temperature, we used chromatin immunopurification (ChIP) to follow H2A.Z occupancy at the *HSP70* promoter in response to different ambient temperatures. We used *HTA11:GFP* expressed under the endogenous *HTA11* promoter for our ChIP experiments. This construct complements the *hta9 hta11* double mutant (Figure 4A), showing that *HTA11:GFP* is functional. Microscopy

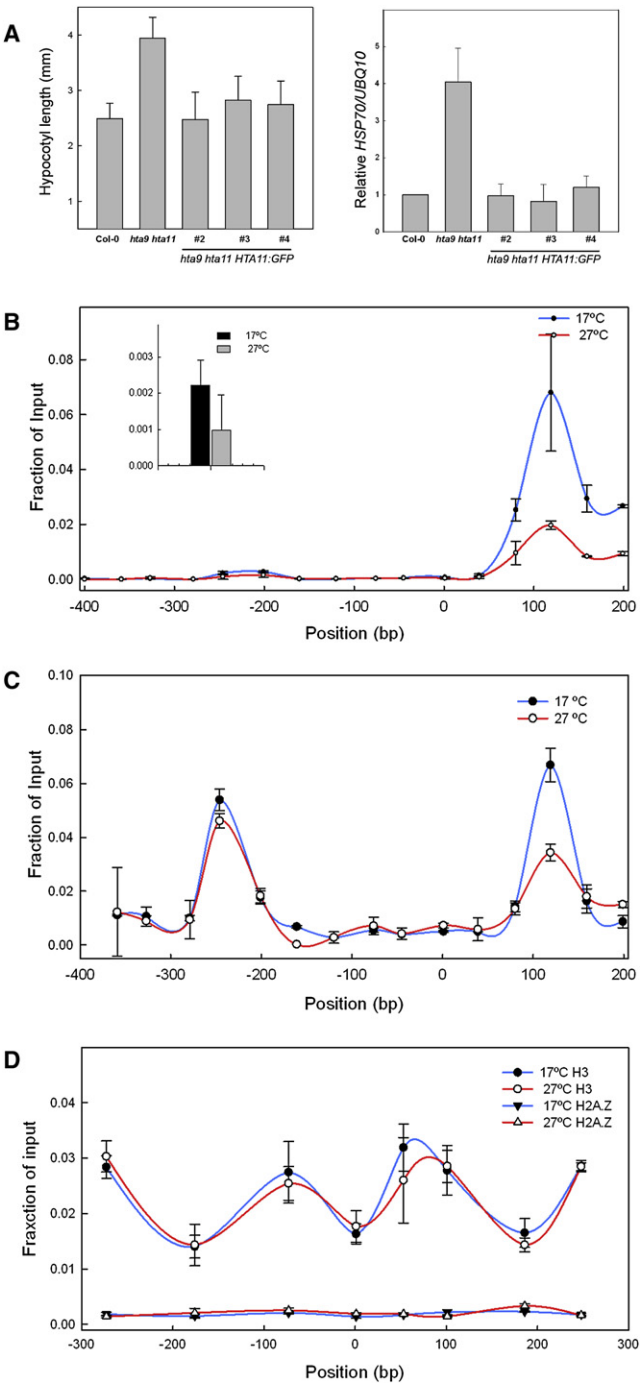

Figure 4. H2A.Z Occupancy Varies as a Function of Ambient Temperature

(A) *HTA11:GFP* expressed under the native promoter is functional and complements the *hta9 hta11* double mutant. The *hta9 hta11* mutant has an extended hypocotyl compared to Col-0 at 21°C. This phenotype was completely rescued in three independent lines expressing *HTA11:GFP* under the native promoter (left panel). Error bars represent standard deviation (SD) from 20 seedlings from each genotype. *HTA11:GFP* also complements the elevated *HSP70* expression in the *hta9 hta11* double mutant (right panel). Values are mean ± SD.

(B) Enrichment of H2A.Z at the *HSP70* promoter was analyzed using *HTA11:GFP*. Formaldehyde-crosslinked chromatin from 7-day-old seedlings grown at 17°C (blue) or after 2 hr of incubation at 27°C (red) was digested with MNase and was used for chromatin immunopurification (ChIP) analyses. H2A.Z immunocomplex-associated DNA fragments were quantitatively analyzed using tiling oligonucleotides (See Figure 4B). A region corresponding to the +1 nucleosome is enriched in H2A.Z where as the −1 nucleosome has a much reduced H2A.Z occupancy (inset bar graph, 4A). x axis: the nucleotide positions across *HSP70* gene with respect to TSS. y axis: H2A.Z occupancy as a fraction of undigested input chromatin. Values are mean ± SD from one representative experiment. Bar graph in the inset shows the −1 nucleosome in detail as represented by the amplicon centered on −246 bp.

(C) ChIP analysis of crosslinked H3 on seedlings treated similarly as above shows the well-positioned nucleosomes of *HSP70* promoter. On temperature shift to 27°C, H3 occupancy changed at the +1 nucleosome where H2A.Z occupancy is high. The relative change in H3 occupancy in response to temperature shift was lower than that of H2A.Z. Values are mean ± SD from one representative experiment.

(D) A gypsy-like transposon gene (At4g07700) acts as a control since it has positioned nucleosomes devoid of H2A.Z and it is transcriptionally inactive and unresponsive to temperature changes. ChIP analysis revealed no change in histone H3 occupancy at both 17°C and 27°C, indicating that H2A.Z confers temperature-specific responsiveness to nucleosomes. As expected, H2A.Z occupancy was absent as measured by ChIP. Values are mean ± SD from one representative experiment.

See also Figure S2.

of HTA11:GFP reveals incorporation into euchromatin as in other studies (Figures S2A and S2B) (Zilberman et al., 2008).

ChIP analysis revealed a greatly depleted H2A.Z occupancy at the *HSP70* promoter in *arp6-10* (Figure S2C), suggesting that the increased *HSP70* expression in *arp6-10* is a consequence of this change in chromatin architecture, as indicated by the *hta9 hta11* phenotype. To see if temperature affects H2A.Z occupancy at *HSP70*, we compared seedlings grown at 17°C with seedlings shifted to 27°C for 2 hr. Consistently, at 17°C, where expression of *HSP70* is low, we observe a high occupancy of H2A.Z at the +1 nucleosome (Figure 4B). This occupancy is, however, very temperature dependent, with H2A.Z occupancy decreasing sharply at 27°C compared to 17°C (Figure 4B). We have validated our ChIP results using an H2A.Z antibody (Deal et al., 2007) that gives similar results (Figure S2D). These results are consistent with a model where H2A.Z occupancy is rate limiting for the transcription of temperature-responsive genes, and the occupancy of H2A.Z responds to temperature. To determine whether the behavior we observe is specific to H2A.Z or is a general property of nucleosomes in response to temperature, we carried out parallel ChIP studies using histone H3-specific antibody. Upon shifting to 27°C, H3 levels at the +1 nucleosome drop, though not to the extent of H2A.Z (Figure 4C). Interestingly at the −1 nucleosome, where H2A.Z occupancy is reduced, H3 levels did not drop significantly. This suggests that H2A.Z-containing nucleosomes exhibit specific dynamic responses to temperature. Since RNA Pol II must negotiate +1 nucleosomes for transcription to occur, this suggests a model where gene transcription may be controlled by the temperature-responsive occupancy of +1 nucleosomes.

The changes we observe in H3 occupancy at *HSP70* may be a consequence of H2A.Z nucleosomes having a temperature-specific response. We therefore extended our analysis to the transcription start site (TSS) of another locus, At4g07700, chosen because it does not contain H2A.Z (Zilberman et al., 2008) and it is largely transcriptionally silent, avoiding complicating factors

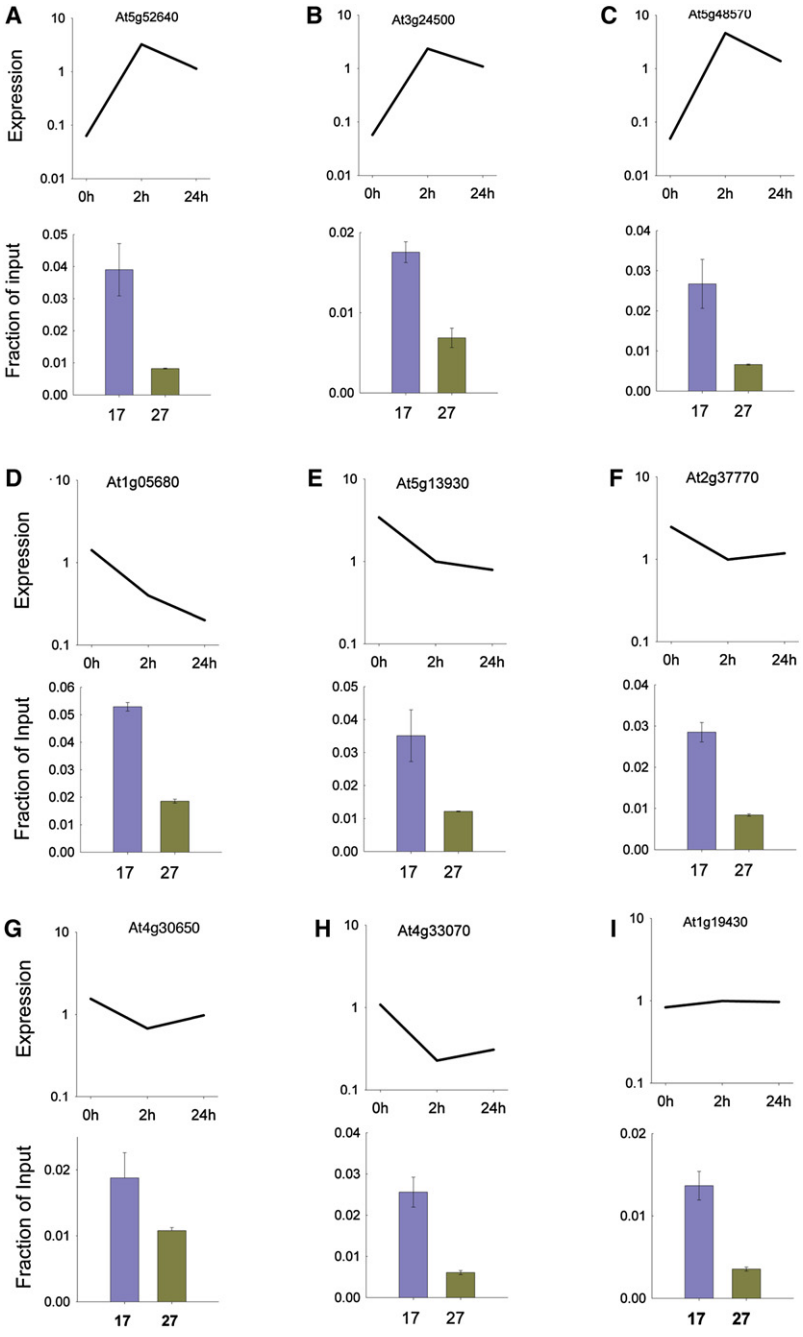

Figure 5. H2A.Z Occupancy Dynamics Are Independent of the Transcriptional Response

Genes with diverse transcriptional response were analyzed for H2A.Z occupancy in response to temperature shift. H2A.Z occupancy is primarily determined by temperature, independent of the specific transcriptional response. This result indicates that H2A.Z-containing nucleosomes confer temperature information on chromatin, which could be interpreted according to the respective regulatory environment for appropriate gene expression. Upper panels show the transcriptional response upon shift to 27°C. 0 hr represents sample from plants grown at 12°C; 2 hr and 24 hr are after shift to 27°C. H2A.Z occupancy dynamics upon shift to 27°C for 2 hr is shown in the lower panel. Values are mean ± SD from one representative experiment.

arising from RNA Pol II-mediated displacement of nucleosomes, as well as being predicted to have a well-ordered nucleosome structure using a recent model (Kaplan et al., 2009). ChIP analysis with H3-specific antibody showed the predicted nucleosome occupancy. ChIP analysis revealed that there was no significant change in H3 occupancy at this locus upon shifting to 27°C (Figure 4D). This is consistent with our findings for the −1 nucleosome of *HSP70* where there is little or no H2A.Z incorporation, and H3 levels do not show significant temperature-responsive dynamics. These results suggest that H2A.Z is the major determinant of temperature responsiveness of chromatin.

To test if this response is unique to *HSP70*, we examined the TSS-proximal regions corresponding to the presumptive +1 nucleosomes of a number of other temperature upregulated genes (Figures 5A, 5B, and 5C). Consistently, all these genes show a sharp reduction in H2A.Z occupancy at 27°C. Because it has been shown that *FT* is an essential output of the thermosensory pathway for mediating early flowering, we sought to see if H2A.Z is present in the *FT* promoter and if it is thermoresponsive. Although *FT* is not significantly expressed in seedlings, eviction of H2A.Z may be necessary to confer transcriptional competence on the *FT* locus. Analysis of global H2A.Z occupancy data (Zilberman et al., 2008) revealed that H2A.Z is enriched in the *FT* promoter. We therefore assayed for H2A.Z changes at *FT* in response to temperature change. Consistently, we observe H2A.Z depletion from the *FT* promoter at higher temperatures (Figure S2E), suggesting an explanation for the acceleration of flowering in *arp6*.

Eviction of H2A.Z Occurs Independently of Transcription

We have shown that H2A.Z occupancy decreases dynamically with increasing temperature at the +1 nucleosomes of temperature-induced genes. Because temperature-dependent histone eviction may be a consequence of greater transcriptional activity, and is not necessarily the cause, we sought to determine if H2A.Z eviction occurs independently of transcription. To test this, we analyzed genes that are enriched for H2A.Z in their promoters and are either downregulated or show constant expression in response to increased temperature. We then performed ChIP to assay H2A.Z occupancy in response to ambient temperature change (Figures 5D, 5E, 5F, 5G, 5H, and 5I). We observed a significant decrease in H2A.Z occupancy across all the genes examined, independent of their transcriptional response to temperature. This result suggests that

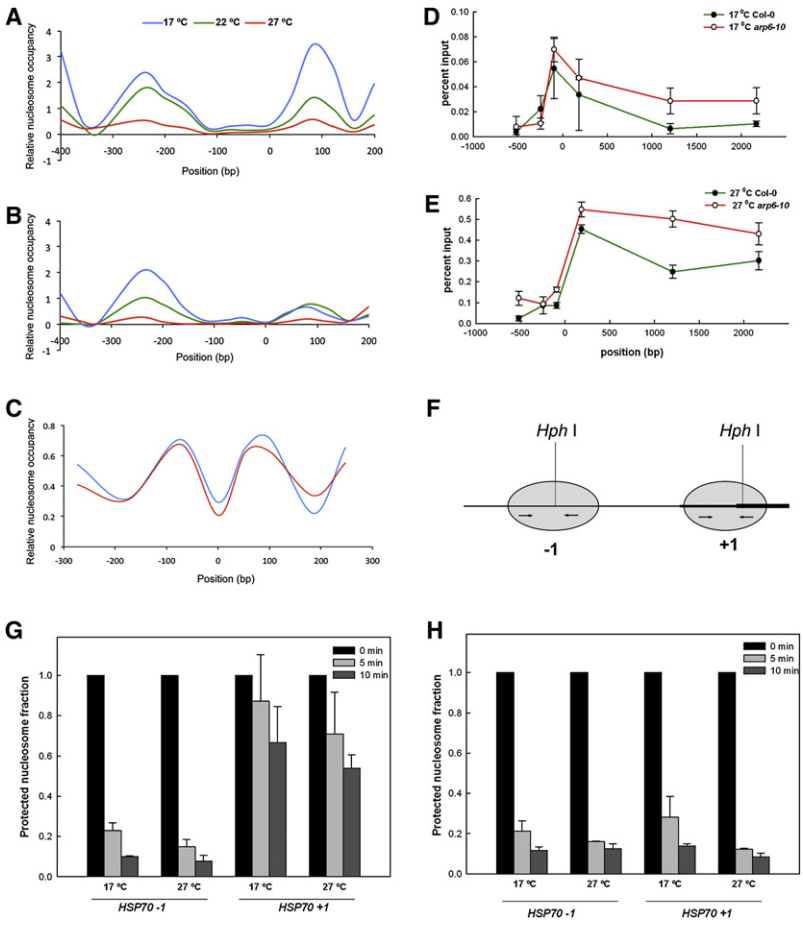

Figure 6. Chromatin Architecture Responds Dynamically to Changes in Ambient Temperature

(A) Chromatin architecture dynamics at *HSP70* in response to temperature in wild-type. Chromatin from 7-day-old seedlings growing at 17°C (blue), 22°C (green), and 27°C (red) was MNase treated and analyzed by qPCR with tiled oligos. The +1 nucleosome of *HSP70* exhibits a robust response to increasing ambient temperature, with nucleosome occupancy and temperature being negatively correlated.

(B) *arp6* shows a constitutive temperature response in terms of chromatin architecture as measured by MNase accessibility. +1 nucleosome occupancy in *arp6* is consistently lower, even at 17°C.

(C) Analysis of nucleosome occupancy dynamics in response to temperature at At4g07700 reveals that consistent with the H3 data, nucleosomes at this locus are nonresponsive to changes in ambient temperature.

ChIP analysis for RNA Pol II at *HSP70* in wild-type (green) and *arp6* (red) grown at 17°C (D) or after 2 hr of incubation at 27°C (E) is shown. x axis represents nucleotide positions on *HSP70* with respect to TSS; y axis represents fraction of input chromatin immunopurified using RNA Pol II antibody. Values are mean ± SD from two experiments.

(F) Schematic representation of HSP70 +1 and −1 nucleosomes showing the HphI sites. Grey ovals represent nucleosomes; oligo positions are indicated by arrows.

(G and H) Temperature-dependent nucleosomal DNA accessibility assay for HphI enzyme in wild-type (G) and *arp6-10* (H). Nucleosomes containing H2A nucleosomes (−1 in wild-type and both −1 and +1 in *arp6-10*) show constitutive accessibility of restriction sites, whereas H2A.Z-containing nucleosomes occlude access. Relative amounts of protected input nucleosomal DNA were calculated and were normalized against the +1 nucleosome of At4g07700 where HphI does not cut. Values are mean ± SD from two experiments.

See also Figure S3.

H2A.Z-containing nucleosomes have a binding affinity or occupancy as measured by ChIP that varies with temperature. This is independent of transcription, which is analogous to the behavior of nucleosomes at the Hsp70 locus of *Drosophila* (Petesch and Lis, 2008). As the presence of H2A.Z-containing nucleosomes is rate limiting for the expression of the majority of the genes in the ambient temperature transcriptome, this suggests that H2A.Z responses to temperature play a key role in integrating temperature information.

The *HSP70* +1 Nucleosome Shows a Constitutive Temperature Response in *arp6*

Because we have found that H2A.Z nucleosomes are evicted at higher temperature, we analyzed the nucleosome structure of the *HSP70* promoter at different temperatures by studying MNase accessibility. MNase digests nucleosome-free DNA and the linker regions between nucleosomes, whereas the DNA of the nucleosomes themselves is protected from digestion (Petesch and Lis, 2008). For plants grown at 17°C, 22°C, and 27°C, we observed a sharp drop in nucleosome occupancy with greater temperature (Figure 6A), which helps us understand how *HSP70* transcription increases with temperature. By comparison, the +1 nucleosome of *HSP70* displays a constitutively open conformation in *arp6.* This is the case even at 17°C (Figure 6B), at which temperature there is significant nucleosome occupancy in wild-type. At higher temperatures, we also observe a decrease in the −1 occupancy, which is likely a consequence of greater transcriptional activity. This decrease in −1 occupancy appears to be H2A.Z independent. Consistently, the nucleosome structure of At4g07700, which does not contain H2A.Z nucleosomes, does not change with temperature (Figure 6C).

The results so far are consistent with a model where expression of *HSP70* may be regulated at the level of nucleosome occupancy. For RNA Pol II to transcribe target genes, it must be able to overcome the physical barrier posed by nucleosomes (Knezetic and Luse, 1986; Lorch et al., 1987).

Numerous studies indicate that nucleosomes at genes induced to undergo transcription dissociate or partially unwrap from their DNA, allowing passage of RNA Pol II, and the presence of nucleosomes, especially the +1 nucleosome near the transcriptional start site, can be rate limiting for controlling transcription (Boeger et al., 2003, 2008). Under heat-shock conditions, nucleosomes at the *Drosophila* Hsp70 locus are lost from the

body of the gene in advance of the arrival of RNA Pol II, indicating that the nucleosomes are able to play a role in regulating transcription (Petesch and Lis, 2008).

RNA Pol II ChIP was used to study transcriptional dynamics at *HSP70* in wild-type and *arp6-10*. At 17°C in wild-type, we observed a greater proportion of RNA Pol II present at the TSS compared to in the body of the gene. By comparison, in *arp6* a greater proportion of RNA Pol II is present in the body of the gene (Figure 6D). At 27°C there is a strong shift of RNA Pol II occupancy, with the highest peak now occurring downstream of the TSS. As this new RNA Pol II peak corresponds to the region occupied by the +1 nucleosome, it is likely that in wild-type at noninductive conditions the +1 nucleosome plays a role in maintaining transcription in a poised state. Upon increasing temperature, the presence of Pol II on the gene body increases considerably (Figure 6E). This is consistent with studies in *C. elegans* and *Drosophila*, which have suggested that H2A.Z +1 nucleosomes may maintain RNA Pol II in a poised state (Mavrich et al., 2008; Whittle et al., 2008). The presence of H2A.Z-containing nucleosomes is therefore rate limiting for the upregulation of *HSP70*.

H2A.Z Nucleosomes Wrap DNA More Tightly

We have found that in the absence of H2A.Z incorporation, canonical H2A-containing nucleosomes are unable to prevent constitutive expression of the warm temperature transcriptome of both up- and downregulated genes. This difference could result directly from a difference in the behavior of H2A.Z nucleosomes or may reflect the effect of other factors recruited by H2A.Z. To distinguish between these two possibilities, we purified nucleosomes from plants grown at 17°C using hydroxyapatite chromatography following a partial MNase digest. It has recently been demonstrated that RNA Pol II does not actively unwrap nucleosomes but depends on fluctuations that locally unwrap DNA for it to transcribe its template (Hodges et al., 2009). Because the invasion of RNA Pol II into nucleosomal DNA is the rate-limiting step for transcription, we assayed the exposure of restriction enzyme sites occluded by H2A.Z and H2A nucleosomes at 17°C and 27°C (Figures 6F and 6G). This assay provides a direct measure of the degree of local unwrapping and exposure of nucleosomal DNA (Polach and Widom, 1995). The H2A-containing −1 nucleosome of *HSP70* displays a greater accessibility as compared to the H2A.Z-containing +1 nucleosome at both 17°C and 27°C. This greater accessibility of H2A-containing nucleosomes provides an explanation for the greater transcription of *HSP70* in the *arp6* background where H2A.Z is not incorporated into chromatin. The rate of exposure of these sites appears to increase with higher temperature, suggesting that local fluctuations of the nucleosomal DNA increase with temperature. Because there might be subtle differences in the accessibility of the HphI site between the −1 and +1 nucleosomes, we purified nucleosomes from *arp6*. In this material both nucleosomes contain H2A, and as we would predict, they are both equally accessible (Figure 6H). These results show that H2A.Z-containing nucleosomes wrap DNA more tightly, and higher temperatures may overcome this. Given that these nucleosomes were purified from plant material, we do not exclude that posttranslational modifications, for example acetylation, may be involved in modulating this response. Importantly, our assay is performed on highly purified material, indicating that the dynamic responses we observe are an inherent property resulting from the nucleosome-DNA interactions. Our finding that the degree of local unwrapping of DNA on H2A.Z-containing nucleosomes is reduced compared to H2A nucleosomes suggests a direct mechanism by which transcription can be adjusted according to the temperature of the cell.

The Role of H2A.Z in Controlling the Temperature Transcriptome Is Conserved

Because temperature changes represent a fundamental challenge to all sessile organisms, we sought to determine if control of temperature transcriptomes might be a conserved function of the H2A.Z nucleosomes in eukaryotes generally. To test this, we analyzed the role of the budding yeast H2A.Z, Htz1, in temperature signaling. The Htz1 transcriptome has been previously determined (Meneghini et al., 2003), and we therefore tested to see if those genes that are misregulated in the *htz1*Δ background also show a corresponding change when wild-type yeast are shifted from 29°C to 33°C (Gasch et al., 2000). Strikingly, we see a significant correlation ($R^2 = 0.30$, $p < 0.001$), with genes that are upregulated at higher temperatures tending to also be upregulated in *htz1*Δ and vice versa (Figure S3). This is the same relationship we have observed in *Arabidopsis* (Figure 3E), showing that the loss of H2A.Z-containing nucleosomes phenocopies cells at higher temperature. These data suggest that warm temperature signals are mediated through H2A.Z nucleosomes in yeast as well as in plants.

DISCUSSION

We have found that the ambient temperature transcriptome is coordinated by H2A.Z-containing nucleosomes in plants. We show that the occupancy of H2A.Z decreases with temperature, and this effect is independent of transcription. in vitro assays show that H2A.Z-containing nucleosomes wrap DNA more tightly than canonical H2A nucleosomes. Consistently, when H2A.Z deposition is prevented, plants display a constitutive warm temperature response, developmentally and transcriptionally, showing that H2A.Z nucleosomes provide information on the thermal status of the cell.

Nucleosomes as Temperature-Responsive Regulators of Transcription

Nucleosomes play an active role in controlling transcriptional processes through modulating the ability of transcription factors to access their *cis* elements or occluding the passage of RNA Pol II (Lam et al., 2008; Segal and Widom, 2009). Access of proteins to DNA wrapped around histones is mediated through local unwrapping events. This unwrapping is not affected by changes in ambient temperature in our study and others for H2A-containing nucleosomes (Polach and Widom, 1995). By contrast, H2A.Z nucleosomes exhibit a much tighter wrapping of their DNA, consistent with studies of nucleosomal stability (Thambirajah et al., 2006). This is supported by the observation that H2A.Z-containing nucleosomes are more tightly positioned in the

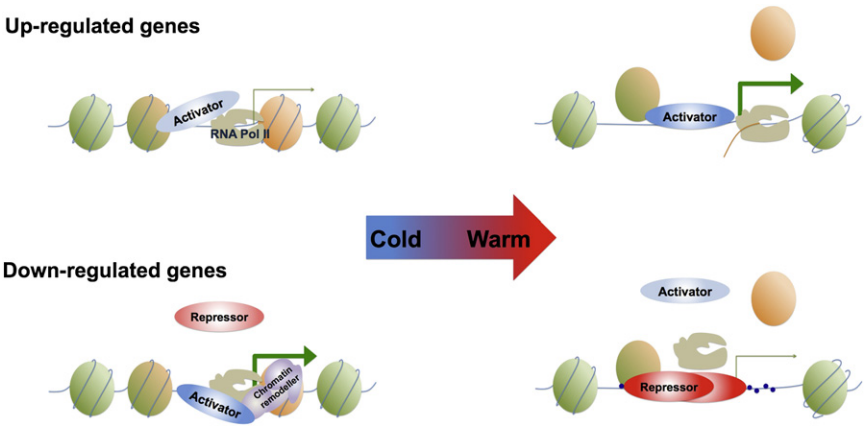

Figure 7. H2A.Z-Containing Nucleosomes Can Modulate Transcription in a Temperature-Dependent Manner

At lower temperature, H2A.Z nucleosomes have a high level of occupancy. H2A.Z nucleosomes may prevent transcription, either by acting as a physical block to the progression of RNA Pol II or by occluding gene-specific *cis*-elements from activating transcription factors. For genes that are specifically expressed at low temperature, H2A.Z occupancy may prevent the binding of repressors or antagonize DNA methylation. A chromatin-remodeling complex may relieve the nucleosomal repression when appropriate components and conditions exist. At higher temperature, H2A.Z nucleosome occupancy declines. This leads to increased expression of genes like *HSP70,* where transcription is limited by H2A.Z occupancy. For genes whose expression is decreased at higher temperature, this loss of H2A.Z may facilitate repressor binding. Conventional H2A nucleosomes are depicted in light green ovals and the degree of H2A.Z occupancy is depicted in orange.

genome and are less fuzzy. Work on reconstituted nucleosomal arrays has also shown that the intranucleosomal interactions are stronger in H2A.Z-containing nucleosomes, whereas these have weaker internucleosomal interactions (Fan et al., 2002). Our results suggest that, in addition to H2A.Z-containing nucleosomes having more tightly wrapped DNA, the degree of unwrapping may also be responsive to temperature. This result suggests a direct mechanism by which temperature may influence gene expression, as it has been shown that RNA Pol II does not actively invade nucleosomes but waits for local unwrapping of DNA from nucleosomes before extending transcription (Hodges et al., 2009). In this way, genes with a paused RNA Pol II will show increased transcription with greater temperature as local unwrapping is increased (Figure 7). For those genes whose transcription decreases with temperature, we propose that greater temperature increases the frequency with which transcriptional repressors may bind. The presence of H2A.Z nucleosomes in these genes may prevent binding of a transcriptional repressor or may antagonize DNA methylation, which has been shown to be a role of H2A.Z in plants (Zilberman et al., 2008). Consistently, H2A.Z-containing nucleosomes have been shown to play an important role in antisilencing (Meneghini et al., 2003).

We cannot rule out that in the *arp6-10* background, a particular transcription factor is misregulated, and this causes some of the transcriptional change we see. This is, however, rather unlikely in view of the large number of genes that are both up- and down-regulated in *arp6-10* and exhibit a corresponding change with increased temperature. Furthermore, we observe the same de-regulation of the warm temperature transcriptome in the *htz1*Δ mutant in yeast, suggesting that this is a conserved function of H2A.Z-containing nucleosomes. Importantly, there are many physiological responses to temperature, and undoubtedly many mechanisms for sensing and responding to temperature in different organisms. For example the PIF4 transcription factor mediates the high temperature architecture phenotypes in plants (Koini et al., 2009). Our mechanism for H2A.Z-containing nucleosomes provides an attractive model for how these transcriptional responses may be integrated with temperature status. On theoretical grounds it has been shown that regulating transcription at multiple levels greatly improves robustness and adaptivity (Tsankov et al., 2006).

Connections between Thermosensing and Flowering Pathways

Flowering is one of the major developmental pathways regulated by ambient temperature. It has been shown that two autonomous flowering pathway genes, *FVE* and *FCA*, are necessary for mediating the ambient temperature response for flowering (Blazquez et al., 2003). Interestingly, an *arp6* allele, *esd1-2*, is epistatic to *fve* and *fca* (Blazquez et al., 2003; Martin-Trillo et al., 2006). *FVE* is a homolog of the mammalian retinoblastoma-associated protein, a component of a histone deacetylase complex (Ausin et al., 2004; Kim et al., 2004). As well as affecting the autonomous pathway, which regulates flowering time through the floral pathway integrator *FLOWERING LOCUS C (FLC), FVE* is also involved in sensing cold temperatures (Kim et al., 2004). Taken together, these data suggest that *FVE* may exert its effects on temperature-dependent pathways through modulating H2A.Z, perhaps through acetylation, which affects nucleosome stability (Ishibashi et al., 2009; Thambirajah et al., 2006). Although the thermosensory flowering pathway is mediated by *FT* expression levels, the major regulator of *FT* expression in response to long photoperiods, *CONSTANS (CO)*, is not essential for perceiving temperature, as *co-1* responds to thermal induction of flowering (Balasubramanian et al., 2006). Our observation that the chromatin status at the *FT* locus responds to temperature and is altered in the absence of H2A.Z provides an explanation for how the thermal induction pathway may activate *FT* expression in response to higher temperature independently of *CO*. Interestingly, another temperature-dependent pathway that also affects flowering time in

plants, vernalization, is also perturbed in the *arp6* background (Choi et al., 2005; Deal et al., 2007). A vernalization requirement in certain *Arabidopsis* genotypes is suppressed by *arp6.* This effect is mediated through *FLC*, a floral repressor whose expression is dependent on the SWR1 complex. Interestingly, in a large field experiment it was found that natural temperature fluctuations were likely to be sufficient for overcoming *FLC*-induced repression of flowering in *Arabidopsis* (Wilczek et al., 2009). Temperature fluctuations of this type are likely to signal through H2A.Z dynamics, which is consistent with the observation that perturbation of H2A.Z in *arp6* is sufficient to overcome a vernalization requirement (Choi et al., 2005; Deal et al., 2005; Martin-Trillo et al., 2006).

Temperature Perception and Climate Change

Although many plants have shown a rapid acceleration of flowering time in response to climate change (Fitter and Fitter, 2002), species that do not are going locally extinct at a high rate (Willis et al., 2008). This indicates that phenological responses to temperature change are of high adaptive value. A molecular understanding of the mechanism of temperature perception will enable us to understand how different species will respond to further increases in temperature and be a key step toward breeding crops able to withstand climate change.

EXPERIMENTAL PROCEDURES

Genetic Screens for Temperature Perception

All experiments were carried out in Col-0 background. A forward genetic screen of fast neutron mutagenized population of *HSP70::LUC* reporter strain was carried out to identify mutants perturbed in the ambient temperature perception pathway (seeds irradiated at HAS KFKI-Atomic Energy Research Institute, Hungary). Seven-day-old seedlings were incubated at 12°C for 48 hr before shifting to 27°C for 3 hr and screening for LUC expression by spraying with Luciferin. The *entr* mutations were identified as having significantly higher LUC induction than the reporter line. *ENTR1* was identified as *ARP6* through a microarray-based transcript analysis. A genomic fragment spanning the *ARP6* promoter and all exons and introns (P_{ARP6}::*ARP6*) was used to complement *entr1*.

Transcript Analysis

Transcript analyses were performed on total RNA extracted using Trizol reagent (Invitrogen). Labeled targets were hybridized to Affymetrix ATH1 array according to the manufacturer's instructions. Microarray data were analyzed using GenespringGX 7.3 (Agilent).

Chromatin Immunopurification

Chromatin immunopurification (ChIP) was performed as described (Gendrel et al., 2002) with minor modifications. Seven-day-old seedlings grown at 17°C were shifted to 27°C for 2 hr and ChIP experiments were performed in parallel on chromatin from both temperatures. For H2A.Z and H3 ChIP, cross-linked chromatin was fragmented with 0.2 units of Micrococcal nuclease (Sigma) in 1 ml of MNase digestion buffer (10 mM Tris-HCl [pH 8.0], 50 mM NaCl, 1 mM β-mercaptoethanol, 0.1% NP40, 1 mM $CaCl_2$, and 1× protease inhibitor cocktail [Roche]). Digestion was stopped using 5 mM EDTA. HTA11, one of the *Arabidopsis* H2A.Z homologs, was GFP tagged and ChIP was performed using GFP polyclonal antibody (Abcam, ab290). Histone H3 dynamics were assayed using H3 antibody (Abcam, ab1791). For RNA Pol II occupancy dynamic experiments, chromatin was fragmented by sonication in lysis buffer (10 mM Tris-HCl [pH 8], 150 mM NaCl, 1 mM EDTA [pH 8], 0.1% deoxycholate, and 1× protease inhibitor cocktail) and ChIP was performed using monoclonal antibody to RNA polymerase II CTD repeat YSPTSPS (Abcam, ab817). All ChIP experiments were performed in buffer containing 10 mM Tris-HCl [pH 8.0], 5 mM EDTA [pH 8.0], 150 mM NaCl, 1% Triton X-100, and 1× protease inhibitor cocktail. Relative enrichment of associated DNA fragments was analyzed by quantitative PCR (qPCR). All oligonucleotide sequences used for target DNA detection and quantification in ChIP experiments are given in Table S1. Each ChIP experiment was repeated at least three times and the data presented are from a representative experiment.

Nucleosome Positioning

Micrococcal nuclease (MNase) digestion of enriched chromatin followed by qPCR using tiled oligonucleotides surrounding TSS was used for nucleosome positioning and analysis. Oligos were designed to have 95–110 bp amplicons every 35–45 bp in the HSP70 promoter. For nucleosome positioning, chromatin from 7-day-old seedlings of Col-0 and *entr1* grown at varying temperatures (17°C, 22°C, 27°C) were digested with MNase, and mononucleosome-sized fragments were gel purified and used in qPCR. Relative nucleosome occupancy was represented as fraction of uncut chromatin DNA and was plotted against the *HSP70* gene position with respect to the TSS for each primer pair where the position denotes the center of each amplicon. Oligonucleotide sequences used are provided in Table S1.

Nucleosome Purification by Hydroxyapatite Chromatography

Seedlings growing at 17°C were frozen in liquid nitrogen without crosslinking and chromatin was prepared as for the ChIP experiments. Following MNase digest for 15 min at 17°C, chromatin fragments were bound to hydroxyapatite and nucleosomes devoid of linker histones and other associated proteins were purified according to Brand et al. (2008). Purified nucleosomes were buffer exchanged to 10 mM Tris-HCl (pH 8.0), 1 mm EDTA (pH 8.0), 25 mM NaCl, and 1× protease inhibitor cocktail. Approximately 500 ng equivalent of nucleosomal DNA was assayed for restriction enzyme accessibility at 17°C and 27°C. The fraction of the input nucleosomal DNA protected was obtained using qPCR for HSP70 −1 and +1 nucleosomes. Data represented are normalized against the +1 nucleosome of At4g07700 that does not have a restriction site.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at doi:10.1016/j.cell.2009.11.006.

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